APPARATUS AND METHODS FOR INFRARED CALORIMETRIC MEASUREMENTS

Cross-References

This application is based upon and claims benefit under 35 U.S.C. § 119 of the following U.S. Provisional Patent Applications, each of which is incorporated herein by reference: Serial No. 60/249,931, filed November 17, 2000; and Serial No. 60/256,852, filed December 19, 2000.

This application is a continuation of U.S. Patent Application Serial No. _____, filed January 17, 2001, titled APPARATUS AND METHODS FOR INFRARED CALORIMETRIC MEASUREMENTS, and naming Andy C. Neilson, Jay S. Teich, Michael R. Sweeney, James D. Orrell III, Marc Samson, John M. Hopkins, and Michael W. Oster as inventors.

Field of the Invention

The invention relates to calorimetry. More particularly, the invention relates to apparatus and methods for performing calorimetry that use optical devices to detect thermal processes and/or multiwell sample plates to support samples for use with such optical devices.

Background of the Invention

Thermodynamics has established the interrelationship between various forms of energy, including heat and work. Moreover, thermodynamics has quantified this interrelationship, showing, for example, that in chemical and physiological processes the difference between the energy of the products and the energy of the reactants is equal to

the heat gained or lost by the system. In an "exothermic" process, this difference is negative, so that the process releases heat to the environment. Conversely, in an "endothermic" process, this difference is positive, so that the process absorbs heat from the environment. Thus, "calorimetry," or the measurement of heat production and/or heat transfer, can be used to determine if a chemical or physiological process is exothermic or endothermic and to estimate the energy produced or consumed.

The measurement of heat production and/or heat transfer in chemical and physiological processes can be quite complicated. Standardly, such measurements are made using a device known as a "bomb calorimeter." This device typically includes a sturdy steel container with a tight lid, immersed in a water bath and provided with electrical leads to detonate a reaction of interest inside the calorimeter. The heat evolved in the reaction is determined by measuring the increase in temperature of the water bath.

Unfortunately, bomb calorimeters are inadequate for the measurement of heat production and/or heat transfer in many areas of chemistry and physiology. For example, the study of processes involving uncommon and/or expensive components may require analysis of samples too small for bomb calorimetry. Similarly, the high-throughput screening of pharmaceutical drug candidate libraries for drug activity may require analysis of too many samples for bomb calorimetry.

The analysis of small samples is especially problematic due to their small heat capacities and large surface-to-volume ratios. Many chemical and physiological processes lead to very small changes in temperature ($<0.05°C$), making their analysis susceptible to environmental contamination. In particular, whenever there is a

temperature difference between a sample and the environment, heat can be exchanged between the sample and the environment, for example, by conduction, convection, and/or radiation, among others. Such heat exchange may quickly alter the temperature of a small sample and thereby obscure any temperature change associated with a reaction. Moreover, fluid samples such as those typically used in studies of chemical and physiological processes may initiate secondary reactions with the environment, such as evaporation. Evaporation, by definition, is an exchange of energy (moisture is added to the air, while chemical volume is reduced). This process takes place on the surface of the sample, where the sample is exposed to the environment, and so may be especially problematic for small samples due to their relatively large surface-to-volume ratios. Evaporation not only removes energy from the sample, contaminating the measurement, but also may increase measurement noise due to surface instability as the fluid phase changes to a gas phase.

Summary of the Invention

The invention provides apparatus and methods for performing calorimetry. The apparatus include optical devices for detecting thermal processes and multiwell sample plates for supporting samples for use with such optical devices. The methods include measurement strategies and data processing techniques for reducing noise in measurements of thermal processes. The apparatus and methods may be particularly suitable for extracting thermal data from small differential measurements made using an infrared camera and for monitoring chemical and physiological processes.

Brief Description of the Drawings

Figure 1 is a partially schematic cross-sectional view of a system for detecting thermal processes.

Figure 2 is a top view of a multiwell sample holder for use with an optical device for detecting thermal processes.

Figure 3 is a cross-sectional view of the multiwell sample holder of Figure 2, taken generally alone line 3-3 in Figure 2.

Figure 4 is a graph showing the infrared transmissivity of a preferred sample well material as a function of the thickness of the material.

Figure 5 is a pseudo-color image showing the extent and effect of thermal cross talk in sample wells in (A) the microplate of Figures 2 and 3 and (B) a standard commercial microplate.

Figure 6 is a graph showing noise envelopes associated with measurements of sample temperature taken from the (A) top and (B) bottom of a sample well after application of common-mode noise subtraction, area averaging, and frame averaging. The noise envelope associated with the top-read data is significantly larger than the noise envelope associated with the bottom-read data due to evaporation.

Figure 7 is a graph showing the size of the noise envelope as a function of the number of frames average in an image-averaging experiment.

Figure 8 is a graph showing the effects of common-mode noise and drift on thermal data collected using an infrared camera.

Figure 9 is a graph showing the effects of offset subtraction on thermal data.

Figure 10 is a graph showing the effects of removing common-mode noise on thermal data.

Figure 11 is a software screen for use in the display of thermal data, including temperatures and temperature differentials.

Figure 12 is a software screen for use in the collection and/or analysis of data from measurement and reference regions.

Figure 13 is a software screen for use in defining selected characteristics of the reference region.

Definitions

Technical terms used in this application have the meanings that are commonly recognized by those skilled in the art. The following terms may have additional meanings, as described below:

Common-mode noise. Typically low-frequency (<1 Hz) noise caused when internal control loops, such as the servo on a cryogenic cooler, create response changes in the detector. In an infrared camera, these noise sources may be common to each sensor element and may be geometrically displaced across the sensor array. For example, at a given time, a thermal wave from the expansion of helium gas in a sensor cooler may cause slight gain changes in the sensor that cause a group of sensor elements in one geometric region of the array to respond differently, or out of phase with, another group of sensor elements in another region of the array. In most applications, common-mode noise is insignificant; however, in high-sensitivity (<0.05°C) applications, common-mode noise may become a limiting factor.

Heat. A form of energy associated with the motion of atoms or molecules. Heat is capable of being transmitted by (1) conduction through solid and fluid media, (2) convection through fluid media, and (3) radiation through empty space.

Infrared (IR) radiation. Invisible electromagnetic radiation having wavelengths from about 700 nanometers, just longer than red in the visible spectrum, to about 1 millimeter, just shorter than microwave radiation. Infrared radiation includes (A) near IR (from about 700 nm to about 1,300 nm), (B) middle IR (from about 1,300 nm to about 3,000 nm), and (C) far or thermal IR (from about 3,000 nm to about 1 mm). Near and middle IR is infrared radiation that typically is caused by vibrations and low-level electronic transitions in molecules and that is only peripherally related to heat. In contrast, thermal IR (or thermal infrared radiation) is infrared radiation that is caused or produced by heat and that is emitted by an object in proportion to the temperature and emissivity of the object.

Radiosity. The radiation emanating from an object is determined by the following parameters: (1) emissivity, i.e., the amount of radiation the object emits, (2) reflectivity, i.e., the amount of externally derived radiation the object reflects, and (3) transmissivity, i.e., the amount of externally derived radiation the object transmits. For example, the thermal power P radiated by an object may be described by the equation $P = \varepsilon\sigma AT^4$, where ε is the emissivity of the object, σ is the Stefan-Boltzmann constant, A is the area of the object, and T is the temperature. Emissivity, reflectivity, and transmissivity are dimensionless parameters with values that range between 0 and 1. For a given material, the sum of these parameters should equal unity, so that each parameter

is inversely correlated with the sum of the other parameters. A material with an opaque surface has a transmissivity of zero, so its emissivity equals one minus its reflectivity. Materials that radiate very well and absorb a large percentage of the radiation that strikes them have high emissivities.

Parasitic noise. Typically low-frequency (<0.1 Hz) noise caused by stray radiation incident on the detector from within the detector housing, creating an offset in output that results in measurement error. The stray radiation may be caused by slight temperature changes internal and/or external to the detector. In an infrared camera, the error may be geometrically displaced across the camera array, as determined by the efficiency of the cold shield for the camera sensor and the baffling within the Dewar. Most infrared cameras attempt to correct for parasitic noise using some form of internal calibration mechanism, such as a uniform-temperature shutter that periodically drops in front of the sensor to perform an offset compensation. These calibration mechanisms inherently interrupt measurements and can lead to measurement errors if the uniform-temperature shutter is not actually perfectly uniform in temperature. All infrared radiometers have some form of parasitic noise.

Spatial Noise. Typically lower-frequency (<60 Hz) highly nonlinear noise reflecting detector artifacts caused by variations in the manufacturing process, for example, during metal oxide vapor deposition (MOVD). In an infrared camera, these artifacts may cause slight differences in the gain or response characteristics, spectral characteristics, and/or stability characteristics of the various elements, columns, and/or rows of the sensor. Spatial noise may result in low-frequency noise or a drift component,

which may still remain even after performing a calibration for pixel gain and offset in the camera.

Temporal noise. Typically high-frequency (>60 Hz) random noise caused by (radiated or conducted) electronic noise, A/D quantization, 1/f noise, microphonics, and/or a low electronic signal-to-noise ratio from the detector.

Thermal conductivity. The quantity of heat transmitted, due to a unit temperature gradient, in unit time under steady conditions in a direction normal to a surface of unit area, when the heat transfer is dependent only on the temperature gradient.

Thermodynamic noise. Noise caused by thermodynamic instabilities in a medium, such as a fluid-to-gas phase transition. Surface measurements of most fluids, including water, show significant instability due to thermodynamic noise caused by evaporation.

Detailed Description

The invention provides apparatus and methods for performing calorimetry (or thermogenic analysis). The apparatus include optical devices for detecting thermal processes and multiwell sample plates for supporting samples for use with such optical devices. The methods include measurement strategies and data processing techniques for reducing noise in measurements of thermal processes. The apparatus and methods may be particularly suitable for (1) extracting thermal data from small differential measurements made using an infrared camera, and (2) for monitoring chemical and physiological processes.

Figure 1 shows a system 100 for detecting thermal processes in accordance with aspects of the invention. The system includes an optical device 102 configured to detect thermal radiation 104 and a sample plate 106 having a sample well 107 configured to support a sample 108 for use with the optical device. The system may be used to monitor thermal processes in the sample or samples by detecting temperature changes correlated with heat production (e.g., from a chemical or physiological reaction) and/or heat transfer in the samples. This correlation may be performed using any suitable method, such as those described in the following U.S. provisional patent application, which is incorporated herein by reference: Serial No. 60/256,852, filed December 19, 2000. If there are multiple samples, the radiation transmitted from the samples may be detected sequentially from each sample, for example, by point reading, or simultaneously from some or all of the samples, for example, by image reading. The optical device may include a detector 110 such as an infrared optical sensor configured preferentially to detect thermal infrared radiation. The detector measures thermal energy radiated from a sample (or samples) supported by the sample plate and converts the measured energy to a signal such as an electrical signal that can be converted into a temperature, for example, using a blackbody or graybody approximation. In a preferred embodiment, the detector includes an imaging device such as an infrared focal plane array (FPA) configured to obtain a time-dependent series of two-dimensional infrared images of processes occurring in samples in a multiwell sample plate, permitting measurement of temperature and temperature changes in each process, as a function of time, geometrically across the plate. The series of images typically is collected at a preselected frequency (typically >1 Hz) for

a preselected period significant relative to a characteristic time of any time-dependent process being monitored. The data subsequently may be processed and/or reported at a lower frequency. The data may be used to monitor, screen, rank, and/or otherwise analyze thermal processes occurring in the sample. The thermal analysis may be used alone or together with other measurements to assess the presence, concentration, physical properties, and/or activity of a compound or compounds in the sample. Thus, the system provides a noncontact, noninvasive method for measuring thermal properties such as temperature, in contrast to bomb calorimeters, thermometers, and capacitive and resistive circuits.

The system and its components may be configured to improve the accuracy and/or sensitivity of thermal measurements, particularly thermal measurements involving small samples and/or small temperature changes. The optical device may be configured to reduce measurement errors associated with noise, such as common-mode, parasitic, spatial, temporal, and/or thermodynamic noise, among others. The sample plate may be configured to facilitate detection of thermal radiation through a surface of the plate and/or to reduce measurement-contaminating heat transfer between the samples and the environment (including other samples). The optical device and sample plate may together be configured to reduce noise associated with evaporation, for example, by using a bottom-read detector and a sample plate having an infrared-transmissive bottom surface and in some cases a cover.

The remainder of the Detailed Description is divided into four sections: (A) optical devices, (B) noise reduction, (C) sample holders, and (D) examples.

A. Optical Devices

The optical device generally comprises any device capable of preferentially detecting thermal infrared radiation and using the detected radiation to analyze thermal processes in a sample. The phrase "capable of preferentially detecting thermal infrared radiation" means that the device is configured and/or operated so that it detects more thermal infrared radiation than any other form of radiation (i.e., so that at least about half of the radiation detected is thermal infrared radiation). The phrase excludes any device that detects thermal infrared radiation only incidentally, as might occur in an optical device configured to detect visible light if thermal radiation leaked into the detector. The capability for preferentially detecting thermal infrared radiation may reflect use of one or more of the following mechanisms, among others: (A) use of spectral filters preferentially to "extract" thermal infrared radiating by blocking radiation other than thermal infrared radiation, including visible, near IR, or middle IR radiation, (B) use of detectors having enhanced sensitivity for thermal infrared radiation, and/or (C) postprocessing of a detector signal to reduce and/or compensate for any component of the signal not resulting from detection of thermal infrared radiation.

Figure 1 shows an optical device 102 constructed in accordance with aspects of the invention in use as a part of a system 100 for detecting thermal processes. The device includes a detector 110 configured to detect thermal infrared radiation emitted by a sample, a stage 112 configured to support a sample in a sample holder for thermal analysis by the detector, and a processor 114 configured to analyze radiation detected by the detector. The detector and stage are positioned such that at least a portion of the

thermal infrared radiation emitted by the sample is incident, indirectly or preferably directly, on the detector. This may be accomplished by ensuring that a central axis CA of the sample wells is aligned with an optical axis OA of the instrument prior to detection of thermal infrared radiation. Alignment simply means that the two axes are sufficiently close to parallel that radiation from a central portion of the sample well is detectable by the instrument. For example, in Figure 1, the central axis of each sample well is aligned with the optical axis of the instrument. The detector may be positioned below the stage to form a "bottom-read" instrument, above the stage to form a "top-read" instrument, or in other positions to form other instruments. The instrument of Figure 1 is a bottom-read instrument, and the instrument of Figure 1 with components of the optical device 102 inverted and positioned above the stage is a top-read instrument.

The stage may be movable, so that samples may be deposited at a first position, moved to a second position for fluid dispensing, moved to a third position for thermal equilibration, moved to a fourth position for thermal detection, and moved to a fifth position for pickup. These positions may be the same or different, and any given position (except the detection position) may be present or absent. The stage may move sample holders translationally and/or rotationally, among others.

The sample generally comprises any object or system of objects intended for thermal analysis. The sample may include compounds, mixtures, surfaces, solutions, emulsions, suspensions, cell cultures, fermentation cultures, cells, tissues, secretions, and/or derivatives and/or extracts thereof. The sample also may include the contents of a single sample site, or several sample sites, depending on the assay.

The detector generally comprises any device for preferentially detecting thermal infrared radiation and converting the detected radiation into a signal representative of the detected radiation. A preferred detector is an imaging detector, such as an infrared camera, that is capable of simultaneously viewing part or all of a sample holder.

The stage generally comprises any mechanism for supporting a sample in a sample holder at an examination site for thermal analysis by the device. A preferred stage is a transporter capable of moving the sample holder vertically and/or horizontally between the examination site and one or more transfer sites where the sample holder can be loaded onto and/or unloaded from the stage.

The processor generally comprises any mechanism for analyzing the signal from the detector, for example, to conduct a thermal analysis. The analysis may include conversion of a signal representative of intensity and/or wavelength into a signal representative of temperature and/or differential temperature, among others. The analysis also may include performing calculations to reduce noise and/or to facilitate data reporting, as described below. The processor may be intrinsic to the detector, extrinsic to the detector, or both.

The optical device also may include a housing 116 to support and protect the detector. The housing may include, among others, an optics tube 118, an infrared-transmissive window 120, and/or a baffle 122 having an aperture 124. The optics tube may support the detector and/or components of the housing, such as the window and baffles. The optics tube also may reduce the amount of unintended thermal infrared radiation entering the detector. The window may permit thermal infrared radiation to

enter the housing for detection, while also permitting the housing to be sealed to reduce contamination and/or (partially) evacuated to reduce absorption and/or scattering of thermal radiation prior to detection. The window may include a portion formed of zinc selenide (ZnSe) and/or polyethylene, among others. The baffles may block stray thermal infrared radiation. The optical device may be configured to shield the sample from incident radiation to reduce the proportion of the sample signal arising from transmission, reflection, and/or photoluminescence from the sample.

The optical device also may include a data output mechanism such as a view screen or printer for reporting results of any thermal analysis. Data generally may be reported using any suitable method, physical and/or electronic, digital and/or analog, and static and/or time varying, among others. Suitable methods include tables, graphs, and/or images, among others. Data may include temperatures and/or temperature differentials, among others, at a fixed time or as a function of time.

B. Noise Reduction

Noise is almost invariably a problem in measurements of thermal processes. However, noise may be especially problematic in measurements of thermal processes involving small samples and/or small temperature changes, where even minor noise can obscure or overwhelm any temperature change associated with the thermal process.

The effects of noise on measurements of thermal processes can be reduced using noise reduction techniques. Noise reduction involves identifying sources of noise (e.g., measurement noise, camera noise, etc.) and then developing methods for reducing or eliminating the noise or its effects. Unfortunately, thermal detectors such as infrared

cameras are susceptible to several types of noise, including common-mode, parasitic, spatial, and temporal noise, among others. In addition, fluid samples are susceptible to other types of noise, including thermodynamic noise, which is caused by thermodynamic transformations, such as evaporation, in which the sample changes from a fluid to a gas. Thus, noise reduction in measurements of thermal processes may involve the application of one or more different methods.

The present "state of the art" for infrared, radiometric cameras defines measurement performance in terms of noise-related parameters, specifically, accuracy and sensitivity. Here, accuracy is a relative absence of error or mistake, and sensitivity is an ability to detect or measure an input, especially a weak input. The sensitivity of infrared cameras normally is quantified in terms of noise equivalent temperature difference (NETD), which is the RMS noise/response at a given temperature, f#, and operating frequency (normally 30°C, f/1, and 60 Hz). NETD typically is the limiting factor in determining measurement sensitivity. A typical, state-of-the-art, high-performance infrared camera, such as the FLIR SC3000, using Quantum Well (QWIP) sensor technology, specifies an absolute accuracy of about 2°C and a sensitivity (NETD) of about 0.03°C. Unfortunately, this sensitivity may be inadequate in measurements of small temperature changes.

The invention provides methods for reducing noise and/or enhancing accuracy and/or sensitivity in thermal measurements, particularly measurements involving small samples and/or small temperature changes. These methods may be implemented using any suitable apparatus, such as any processor associated intrinsically or extrinsically with

the optical device. These methods may improve upon the previous state-of-the-art sensitivity described above, potentially providing sensitivities of <0.01°C and RMS noise levels of <0.005°C, at least when the methods are used on data collected with preferred sample holders. The methods may involve application of one or more of the following techniques, among others:

1. Low-pass filtering

Data may be collected at a relatively high frame rate and passed through a low-pass (time-domain) filter to reduce high-frequency "temporal noise." For example, using an infrared camera as described above, full-field radiometric data may be collected at a 60 Hz frame rate and passed through a low-pass filter internal to the camera electronics to yield filtered data corresponding to a lower frame rate.

2. Frame averaging

Data may be averaged (or otherwise smoothed) over a series of frames to remove any residual high-frequency temporal noise. For example, frame averaging may be performed pixel-by-pixel by summing the values T_{ij} associated with a given pixel ij in each frame k and then dividing the sum by the total number N of frames used in the average:

$$\langle T_{ij} \rangle_{FA} = \frac{1}{N} \sum_{k=1}^{N} T_{ij}(k) \quad (1)$$

Here, ⟨⟩ denotes averaging. Typically, the middle frame in the set of N frames is replaced by the average frame. The preferred number of frames to use in the frame average is

determined by competing factors. Generally, it is better to use a larger number of frames because frame averaging and low-pass filtering typically reduce high-frequency random temporal noise by the square root of the number of frames averaged. However, the overall time corresponding to the number of frames used in the average should be small relative to the time scale of thermal changes in the system to avoid averaging frames that differ due to actual differences in the temperature of the sample rather than due merely to noise. In the data presented below under Examples, the optimum number of frames for frame averaging was between about 4 and 16. Frame averaging may be performed separately for measurement and reference areas.

3. **Area averaging**

Data may be spatially averaged to return a reduced number of (average) values or a single (average) value for each area as a function of time. For example, area averaging may be performed by summing the values T_{ij} associated with some or all of the pixels in a given area A of a frame and then dividing the sum by the total number M of pixels used in the average:

$$\langle T \rangle_{AA}(k) = \frac{1}{M} \sum_{i,j \in A} T_{ij}(k) \tag{2}$$

Thus, in a sample holder having 96 sample wells each having a measurement area and a reference area, area averaging may be used to reduce the data set to as few as 96 measurement values and 96 reference values by independently averaging pixel values over all or part of each measurement and reference area. The measurement and reference areas may be distinguished using application software implemented in the processor.

Area averaging typically involves >4 pixel elements and preferably involves >9 pixel elements. Area averaging may reduce the effects of geometric, spatial noise common to most FPA detectors. Such noise may reflect defective or nonlinear detector elements and/or slight differences in amplifier characteristics.

4. Reference calibration

Data may be calibrated using a reference standard, such as an adjacent local (e.g., perimeter) reference standard, for example, by subtracting a reference value from a corresponding measurement value to return a differential measurement for each sample well as a function of time.

$$T_{\mathrm{RC}} = T_{\mathrm{Meas}} - T_{\mathrm{Ref}} \tag{3}$$

Here, the measured and reference values may be properties of the thermal radiation detected from the measurement and reference regions, respectively, such as intensities, or they may be quantities derived from such properties, such as temperatures. The method may be applied pixel-by-pixel or area-by-area, among others. Subtracting reference values from measurement values may reduce or eliminate common-mode noise, internal drift, and/or parasitic noise local to the region of the detector array used in the measurements. These noise sources have a tendency to be geometrically dispersed across the sample holder or sample wells, so that other noise-reduction techniques, such as single-point reference or Fourier transform characterization and subtraction have limited success. These other methods have a tendency to amplify noise where it shifts out of

phase relative to adjacent areas, whereas the local reference compensates for geometric shifting.

5. Offset subtraction

Data may be adjusted by subtracting one or more offsets from each measurement.

$$T_{OS} = T - T_{Offset} \quad (4)$$

The offset may be used to set the initial-time differential measurements for each sample well at t (time) = 0, so that there is a common starting point from which to measure changes in temperature. Offset subtraction effectively creates a zero reference at the beginning of the experiment and adjusts the difference in temperature between the measurement region and associated reference region to zero. Adjusting the offset to zero may compensate for field nonuniformity resulting from camera drift prior to the start of data collection.

6. Bottom reading

Reading through the bottom of an infrared-transmissive sample well may reduce thermodynamic noise created at the interface of dry air and the sample. In particular, evaporation at sample surfaces exposed to dry air may create a saturated gas layer adjacent the sample surface. This layer may be opaque or nearly opaque to the thermal detector and show significant instability (measured to be >0.05°C). Measurement noise created by evaporation may be fivefold or more greater than measurement noise associated with reading through the bottom of the sample well or from an independent black body reference. Additionally, evaporation at the surface may lower the surface

temperatures measured by the camera by as much as 2°C. This 2°C difference is a heat sink for the reaction being measured. Bottom reading allows the top surface of the sample well to be sealed so that the space above the sample becomes saturated with moisture, reducing evaporation noise and heat loss.

The application of these noise-reduction methods generally is quite flexible. For example, each method generally may be applied separately, alone or in combination with any number of other methods. Moreover, each method generally may be applied in any order.

B. Sample Holders

The sample holder or sample plate generally comprises any substrate or material capable of supporting a sample for thermal analysis. Suitable sample holders may include microplates, PCR plates, biochips, chromatography plates, and microscope slides, among others, where microplate wells and biochip array sites comprise assay or measurement sites.

The sample holder may include a thermal isolation structure disposed between the sample wells to reduce thermal transfer between the wells and the environment and thus between adjacent wells. The thermal isolation structure may include a thermal buffer, thermal barrier, and/or isolation well, among others, as described below. The thermal isolation structure may be composed at least in part of a different material than the sample wells. The thermal isolation structure may substantially surround a central or optical axis of each sample well, isolating the wells without obstructing transmission of thermal infrared radiation along the central axis. The thermal isolation structure also may

be disposed such that any straight line below a plane formed by the tops of the sample wells connecting a portion of one sample well to a portion of an adjacent sample well intersects the isolation structure.

The sample holder also may include an insert member defining an array of sample wells and a support member having a thermal isolation framework in a configuration corresponding to the array of sample wells. The sample wells each may have a central axis, and the insert may engage the support member such that each sample well is thermally isolated from adjacent sample wells without obstructing the transmission of thermal infrared radiation along the central axis. The thermal isolation framework may include a thermal buffer, thermal barrier, and/or isolation well, among others, as described below.

The sample holder also may include an insert having a plurality of sample wells, and a thermal isolation member for supporting the insert so that each sample well can be precisely positioned along an optical path, where the thermal isolation member provides a thermally controlled thermal reference surface adjacent each well as viewed along the optical path. The reference surface may define an aperture that frames the associated optical path.

A preferred sample holder is configured as a microplate having a frame and a plurality of sample wells disposed in the frame for holding a corresponding plurality of samples for analysis. This format may combine small-volume samples and a high-density holder, permitting automated analysis of large numbers of samples. This format also may be configured to reduce unintended heat exchange between the samples and the

environment (including between the sample and other samples) and/or to permit an optical detector to measure thermal infrared radiation transmitted through a surface of the sample holder.

The sample holder may include one or more of the following features, among others:

1. **Thin surface**

A sample well having at least one surface having a thickness of less than about 0.005 inches, and preferably less than about 0.001 inches, and most preferably less than about 0.0005 inches. A thin surface may be important for at least two reasons: (1) increased infrared transmissivity, and (2) decreased thermal conductivity. These two criteria preferably may be met using a single material, such as a polymeric polyethylene blend having a high infrared transmissivity (e.g., greater than about 50% or about 80%) and a low thermal conductivity (e.g., less than about 1 W/m-K or about 0.6 W/m-K).

A thin (i.e., reduced-thickness) surface may increase transmissivity. A thin surface may be less likely to absorb thermal energy being radiated by the sample due to its shorter path length and more likely to have an outer (i.e., non-sample-contacting) surface at the same temperature as the sample, facilitating calorimetric analysis through the surface. A preferred thin surface has a high transmissivity (e.g., >80%) for thermal infrared radiation, particularly thermal infrared radiation having wavelengths between about 3 and 5 micrometers and between about 7 and 14 micrometers. (These wavelength ranges may be especially useful in thermal imaging, because they correspond to minima in atmospheric absorption.) A thin more transmissive surface preferably is located at least

at the bottom of the sample well to permit detection from the underside of the sample holder using a bottom-read analyzer. The surface may be substantially (e.g., optically) flat to reduce optical aberrations during analysis through the surface.

A thin (i.e., reduced-thickness) surface also may decrease thermal conductivity. There are three primary mechanisms for heat transfer in the plate: conduction, convection, and radiation. Typically, conduction is the most significant mechanism, and radiation is the least significant mechanism. Conduction may be described by the equation $P = KA\nabla T$, where K is the thermal conductivity, A is the surface area, and ∇T is the temperature gradient. Thus, reducing surface area may reduce conduction. A primary path for conduction is through the walls of the sample well to contact points on the associated frame. This path may be reduced using thin-walled sample wells. Moreover, because the thermal conductivity of air (~0.02W/m-K) is less than that of the preferred well material (0.6 W/m-K), it is important to use the air as much as possible for a conduction path. Thus, the wells hold heat much like a thermos. Finally, the thermal capacitance of a thin material is lower, so that there is less change in temperature due to the initial ΔT in the system. In particular, the material may be selected such that the thermal mass of the sample wells is no more than about half the thermal mass of an aqueous sample positioned in the sample well, even when the sample well is completely full. A thin less conductive surface preferably is located at least at the sides of the sample well..

2. Thermal buffer

A thermal buffer disposed between the sample wells to resist thermal transfer between sample wells, or between the environment and the sample wells. The thermal buffer generally comprises any mechanism for resisting a change in temperature. In this sense, the thermal buffer resembles a pH buffer, which resists a change in pH when an acid or base is added to a solution by binding to the added species, or an electrical capacitor, which resists a change in voltage by storing or releasing charge. The thermal buffer may be used to buffer (or keep relatively constant) the temperature of any structure adjacent the sample well, such as the trapped volume described below. The thermal buffer may include a structure having a high thermal mass (or heat capacity), which can absorb heat without undergoing a significant change in temperature. This high thermal mass structure may, for example, have a substantially higher thermal mass (or heat capacity) than the sample wells and/or corresponding samples, for example, three, five, or even ten times higher. The high thermal mass structure may include a metal such as aluminum and/or a high thermal capacitance plastic, among others.

3. Thermal barrier

A thermal barrier disposed between the sample wells to block thermal transfer between sample wells. The thermal barrier generally comprises any mechanism for blocking the transfer of heat into or out of the sample wells or the vicinity of the sample wells, such as an adjacent trapped volume. The thermal barrier may include a material having a low emissivity and/or a high reflectivity for infrared radiation. For example, the thermal barrier may include a material that reflects at least about half of the infrared

radiation that otherwise would be incident upon surfaces of the sample well. Generally, emissivity and reflectivity are inversely related; thus, shiny, metallic materials tend to have low emissivities and high reflectivities, whereas matte, dark-colored materials tend to have high emissivities and low reflectivities. In a preferred embodiment, the thermal barrier includes a material having a reflectivity of at least about 0.8 and an emissivity of at most about 0.2.

4. Double-walled sample wells

A double-walled sample well, formed, for example, by positioning a sample well in a corresponding isolation well. In a preferred embodiment, a plurality of isolation wells are disposed in a frame, a corresponding plurality of sample wells are disposed in the isolation wells, and none of the sample or isolation wells is in fluid contact with another of the sample or isolation wells. The double-walled wells may include a trapped volume formed between an outer surface of the sample wells and an inner surface of the corresponding isolation wells, further reducing thermal transfer to and from samples positioned in the sample wells. The trapped volume may enclose air and/or an inert gas, and/or be partially or fully evacuated relative to standard atmospheric pressure. The trapped volume also may enclose or be lined along its perimeter with a thermal barrier, i.e., a material having a low emissivity and/or a high reflectivity for infrared radiation to reduce radiation thermal transfer to and from the sample well.

5. Plural optically transmissive surfaces

A plurality of optically transmissive surfaces, at least one associated with the frame and at least one associated with the sample well, where the surfaces are configured

so that an optical reader can detect electromagnetic radiation such as infrared radiation transmitted from a sample through both the optically transmissive surface of the corresponding sample well and the optically transmissive surface of the frame. For example, a plurality of optically transmissive surfaces may be formed by corresponding surfaces of a sample well and isolation well in a double-walled well, as described above.

6. Measurement and reference regions

A combination of a measurement region and a reference region. The measurement region may be a portion of a sample well, and the reference region may be an adjacent portion of the frame, isolated from the sample well, particularly a high-thermal-mass and/or high emissivity (>0.5 and preferably >0.8) surface portion capable of acting as a blackbody or graybody reference. The reference region may be composed at least in part of a different material than the sample wells and may include a metal such as aluminum. The reference region(s) may be disposed adjacent (e.g., about or between) the sample wells, so that each measurement region is near a corresponding reference region, reducing artifacts that reflect temperature drift across the sample plate. Thus, an $M \times N$ array of measurement regions might be complemented by an $M \times N$ of reference regions disposed about the measurement regions, or an $(M-1)\times(N-1)$ array of reference regions disposed between the measurement regions. The reference region may be configured as a ring or annulus distributed about or adjacent a perimeter of the sample well and/or about and preferably symmetrically about a central axis of the sample well. The reference region may be positioned about or above the top of a corresponding sample well, and/or

about or below a corresponding sample well. The reference regions and the corresponding sample wells may be separated by a gap such as an air gap along a line connecting each portion of the thermal reference regions and the corresponding sample wells to reduce heat transfer between the sample wells and the thermal reference regions. Thermal characteristics of the measurement region may be calibrated using thermal characteristics of the reference region, for example, by subtracting the reference characteristic from the measurement characteristic. This calibration may reduce geometrically dispersed common-mode noise, including the effects of internal parasitic radiation and camera drift. The use of dedicated reference regions may free up all of the sample wells for data analysis, because none of the wells needs to be used as a reference well.

7. Consumable sample well inserts

A combination of a reusable frame and a consumable sample well insert (or a set of consumable sample well inserts) configured to fit within or mate with the frame. The combination may facilitate reuse of portions of the sample holder that are expensive, such as the thermal buffer and/or thermal barrier. The combination also may facilitate disposal of portions of the sample holder that contact the sample by reducing the amount of such materials that must be discarded. The combination may be constructed so that the insert is substantially supported by the frame yet substantially thermally insulated or isolated from the frame. The frame and the sample wells may be composed of the same or preferably different materials. Here, consumable may be defined as more likely to be discarded than reused, typically because it is more convenient and/or less expensive to be discarded than

reused. For example, a consumable sample well insert may obviate the need to clean sample wells between samples.

8. Cover

A cover configured for use with the sample holder. The cover generally comprises any mechanism for covering the sample holder, or a portion of the sample holder, to reduce contamination of the samples and/or to reduce evaporation from the samples (e.g., by reducing exposure to dry air and/or convective air currents), among others. The cover generally may be formed of any suitable material, such as a rigid plastic and/or a thin layer of oil or other less evaporative material layered over the sample. The cover may be infrared transmissive, so that samples may be analyzed through the cover using a top-read analyzer. The cover may be configured to touch the top surface of the sample. Alternatively, the cover may be configured to leave an air gap between the top surface and the cover, particularly a small air gap that may quickly saturate with fluid vapor after fluid samples are positioned in the wells and before reactant or catalyst are delivered to reduce evaporative cooling during analysis. Generally, evaporation may be reduced by reducing the size of the air gap, for example, by using shallow wells and/or by substantially filling the wells (for example, until the samples occupy at least about half or even about eight-tenths or nine-tenths of the volume of the sample wells). Alternatively, or in addition, evaporation may be reduced by increasing the humidity of the air adjacent the sample well or sample holder. The cover may include an aperture so that a fluid delivery system such as a pipette can pierce the cover and deliver reactant fluids.

D. Examples

The following examples describe without limitation further aspects of the invention. These examples show that thermal cross talk between sample wells can be reduced by thermally isolating the sample wells and that thermal resolution and the accuracy and sensitivity of thermal measurements can be enhanced by reducing noise, including common-mode, parasitic, spatial, temporal, and/or thermodynamic noise, among others. Additional examples including color drawings showing pseudocolor methods for displaying thermal imaging data are described in the following U.S. provisional patent application, which is incorporated herein by reference: Serial No. 60/256,852, filed December 19, 2000.

Example 1

This example describes a preferred sample holder for use in measurements of thermal processes, including chemical and physiological processes.

Figures 2 and 3 show a sample holder 200 constructed in accordance with aspects of the invention. The sample holder includes a high thermal mass frame or base 202, a plurality of sample wells 204 and a corresponding plurality of windows 206, trapped volumes 208, reference regions 209, and opaque coatings 210, and a cover 212.

Frame 202 is the main structural component of sample holder 200. The frame generally may be sized and shaped as desired, for both convenience and utility. Frame 202 is sized and shaped to form a microplate, enabling the sample holder to be used with standard microplate equipment, such as handlers, washers, and/or readers, among others. A preferred frame is substantially rectangular, with a major dimension X of about

125-130 mm, a minor dimension Y of about 80-90 mm, and a height Z of about 5-15 mm, although other dimensions are possible. Frame 202 may include a base 214 configured to facilitate handling and/or stacking, a notch 216 configured to facilitate receiving the cover, and/or a plurality of apertures 218 configured to receive and support a corresponding plurality of sample wells. The apertures provide clearance around the sample wells, creating an air gap that may provide thermal isolation between the base and the sample well. The apertures may be formed using any suitable method, including machining and/or casting the frame to include the apertures. The inner surface of each aperture may be polished and/or lined with an opaque (i.e., low transmissivity) coating 210 such as AlSiO or gold to reflect infrared radiation and thus to form a thermal barrier to heat conduction to and from the sample wells. In this embodiment, adjacent sample wells may be separated by two thermal barriers and a portion of the frame disposed between the two thermal barriers. The apertures and/or the sample wells may be tapered, such that the separation between the sample wells and the walls of the corresponding apertures increases from the top to the bottom of the sample wells, further reducing conduction between the sample wells and the walls of the apertures.

The frame generally may be constructed of any suitable material. For example, frame 202 is constructed using a material having a high thermal mass (heat capacity) and high thermal conductivity, such as aluminum and/or other metals. Preferred materials such as aluminum may reduce the time required for thermal stabilization within the test chamber while being sturdy enough for repeated, rugged use. In particular, a high thermal

mass base (and/or an adjacent structure) may function as a thermal buffer, helping to maintain a constant temperature around sample wells positioned in apertures 218.

Sample wells 204 are used to support and separate samples 220 for calorimetric analysis. These sample wells may vary in size, shape, number, and arrangement, generally as desired, as long as the wells fit in the frame, and more particularly fit within the corresponding apertures in the frame. Exemplary sizes range between about 1 μL and about 500 μL, and more preferably between about 1 μL and about 200 μL. Exemplary shapes include cones, frustums of cones, cylinders, and parallelepipeds, among others. Exemplary numbers include 96, 384, 864, 1536, 3456, and 9600, among others. Exemplary arrangements include rectangular and hexagonal arrays, among others. Three preferred sample-well configurations that will fit as rectangular arrays within a microplate-sized frame are listed in the following table:

Number of Wells	**Arrangement of Wells**	**Pitch (mm) Between Wells**	**Density (/mm^2) of Wells**
96	8×12	9	1/81
384	16×24	4.5	4/81
1536	32×48	2.25	16/81

Here, pitch is the center-to-center well-to-well spacing, and density is the number of wells per unit area. In a preferred embodiment, the frame will include a similarly spaced array of apertures for receiving the sample wells. A preferred configuration includes 96 frustoconical wells organized in an 8×12 rectangular array. Here, frustoconical refers to a well shape having conical sides and a flat bottom, as shown in Figure 3.

The sample wells may be formed as cups that may be inserted into the frame to hold a sample for calorimetric analysis, permitting an analyzer to measure temperature changes in the sample, for example, resulting from chemical or physiological processes. In this way, a single frame may be used to support cups of different sizes and shapes, so long as the cup (or cups) will fit within the corresponding apertures. The bottom surface 221 of the cup may be flat and/or particularly thin in areas from which infrared measurements are collected. A flat surface may serve to reduce optical distortion and control reflections coming from the surface. A thin surface may enhance infrared transmission, because infrared properties generally are proportional to material thickness. A thin surface also may help to ensure that the outer surface remains at or very close to the temperature of the fluid.

The cup inserts may be formed individually or joined to form a sheet or sheets of cups for use in the frame. Individual cups and small sheets of cups generally provide greater flexibility, permitting cups to be mixed and matched (e.g., according to size, shape, and/or infrared transmission properties, among others) within a single plate. Large sheets of cups provide greater structural stability and convenience, permitting many cups to be changed at once. As mentioned above, cup inserts generally are configured so that a single cup resides within a single aperture in the supporting frame. However, cup inserts also may be configured so that two or more cups reside within a single aperture, permitting a single frame to support cups at two or more significantly different sizes and/or densities.

The material properties of the cups are important for thermal isolation and infrared transmissivity. Figure 4 shows the infrared transmissivity of a preferred cup material as a function of material thickness. This preferred material is an infrared-transmissive (polymeric) polyethylene blend sold under the trademark Poly IR 2™ by Fresnel Technologies. The material has a high infrared transmissivity that increases nonlinearly with decreasing material thickness, showing a significant increase for thicknesses below about 0.001 inches. Moreover, the material has a low coefficient of thermal conductivity (~0.6 watts/meter-kelvin), which is about 1/10 the thermal conductivity of most other infrared-transmitting materials, including Zn, Se, and Ge. In addition, the material has a low thermal mass, so it should quickly assume the temperature of the sample. The low thermal mass of the cup combined with its low thermal conductivity and insulation from the base reduce heat loss to the environment. The infrared transmission properties of the material allow the detector to measure the temperature of the fluid through the cup with less than 10% thermal contribution from the cup. Further aspects of the preferred sample well material are described in the following U.S. provisional patent application, which is incorporated herein by reference: Serial No. 60/256,852, filed December 19, 2000.

Window 206 is an environmental seal between the sample well and the detector(located below the sample holder) when the sample holder is used with a bottom-read analyzer. The window may be formed of an infrared-transmissive membrane material selected to enhance infrared transmission within the spectral sensitivity band of the camera. A preferred material is zinc selenide, which provides >97% transmission to the bottom surface of the sample well.

Trapped volume 208 is formed between inner surfaces of frame 202, sample well 204, and window 206. The high thermal mass frame that surrounds the sample well acts as a capacitor to maintain a constant temperature within the trapped volume, which typically contains air. The inner surfaces of the frame may be lined with an opaque coating, as described above.

Reference region 209 is a source of a reference signal for use in reference calibrations to reduce common-mode and parasitic noise, among others, as described above. Generally, each sample well includes a measurement region and a corresponding reference region. The measurement region generally comprises a portion of the sample or sample well, such as an infrared transmissive bottom portion of the sample well for use with bottom-read instruments. The reference region may comprise an adjacent portion of the frame, such as an annular donut-shaped portion formed around a perimeter and/or central axis of the measurement region. Here, the reference region is positioned at an end of a support member formed by portions of the frame disposed between the sample wells. The thermal mass of the thermal reference region and associated support member may be at least about the same as or greater than the thermal mass of the corresponding sample well and/or sample. The reference region may be formed of a high thermal mass and/or high (>0.8) emissivity material such as a metal that acts as an isolated, blackbody reference. The thermal reference region may include a substantially flat emissive reference surface, where the emissive surface is substantially parallel to a flat portion of the bottom of the sample well and/or where the emissive surface is within a factor of ten of the area of a flat portion of the bottom of the sample well.

Cover 212 provides a mechanism for covering the sample holder, or a portion of the sample holder, to protect samples from evaporation and/or reduce the likelihood and amount of evaporation. Cover 212 generally will leave a small air gap between the sample and the cover that may saturate with fluid vapor to reduce evaporation. Cover includes an aperture 222 so that a fluid delivery system such as a pipette can pierce the cover and deliver reactant fluids.

Example 2

Figure 5 shows results from an experiment designed to measure thermal cross talk caused by heat conduction through the sample plate. The experiment was performed using a top-read thermal-imaging apparatus fitted with a quantum well (QWIP) infrared radiometer from FLIR Systems. The figure shows thermal images of two plates containing a fluid that evaporates when in contact with dry ambient air. Here, relative temperature is denoted by shading, where samples with relatively high temperatures have increased shading, and samples with relatively low temperatures have reduced shading. Plate 1 (left) is fabricated using a thin polymer insert and a high thermal mass base, as shown in Figures 2 and 3. Plate 2 (right) is a standard commercially available 96-well microplate fabricated from a polystyrene polymer (Costar 3628). The thermal images show that plate 1 provides significantly better thermal isolation than plate 2. In particular, the wells in plate 1 are insulated from the surrounding base material, reducing thermal "cross talk" between adjacent wells, whereas the wells in plate 2 are poorly insulated, enhancing thermal "cross talk" and leading to significant thermal gradients across the plate.

Example 3

This example describes results of an experiment designed to test the effects of evaporation on the apparent temperature of samples positioned in wells in a multiwell plate.

The experiment was performed using the top-read thermal-imaging apparatus and low cross-talk multiwell plate of Example 2. In these experiments, sets of adjacent wells were filled with fluids having various evaporation characteristics or else were covered with a high-emissivity tape, as shown below.

T	T	T	T	T	T	T	T	T	T	T	T
T	T	T	W	W	W	A	A	A	T	T	T
T	T	T	W	W	W	A	A	A	T	T	T
T	T	T	W	W	W	A	A	A	T	T	T
T	T	T	O	O	O	W	W	W	T	T	T
T	T	T	O	O	O	W	W	W	T	T	T
T	T	T	O	O	O	W	W	W	T	T	T
T	T	T	T	T	T	T	T	T	T	T	T

Here, A = alcohol, W = water, O = mineral oil, and T = tape. Alcohol and water are prone to evaporation, whereas mineral oil and tape are not. The apparent temperature in each well was measured at fixed intervals during a 15-minute period.

The data show that evaporation affects the apparent temperature of the samples. Specifically, the apparent temperature of wells containing water or alcohol was about 27°C, whereas the apparent temperature of wells containing mineral oil or tape was about 29°C, or about 2°C warmer. Apparently, evaporation of water and alcohol cools the layer of gas above these fluids, leading to lower measured temperatures.

The data also show that evaporation affects the apparent temperature stability of the samples. Specifically, after compensating for common-mode noise, wells containing water or alcohol showed about 0.1°C peak-to-peak (PTP) temperature variations, whereas wells containing mineral oil or tape showed about 0.01°C PTP temperature variations, or about one-tenth as large. Thus, evaporation may preclude accurate measurement of small thermal processes within a well containing a fluid prone to evaporation, at least if measured from above the well. Conversely, reducing evaporation may improve thermal signal and permit a more accurate measurement of thermal reactions within the well.

Example 4

This example describes results of an experiment designed to determine whether temporal noise was caused by evaporation at the surface of the fluid and whether temporal noise could be controlled by reading the fluid through a transparent film.

The experiment was performed using the top-read thermal-imaging apparatus and multiwell plate of Examples 2 and 3. However, here, each well was filled with water. Moreover, a first set of data was collected as above, with the water exposed to ambient air, and a second set of data was collecting after placing a thin (~0.0005-inch thick) infrared-transparent film (Poly IR II) over the wells, in direct contact with the water to simulate a bottom-read design. The temperature in each well was again measured at fixed intervals during a 15-minute period.

Figure 6A ("top read") shows the range of measured temperatures as a function of time, after subtraction of common-mode noise, reading from the surface of the exposed water. The data show significant thermodynamic noise, resulting from evaporation at the

surface of the water, with temperature variations of greater than about 0.1°C (PTP). This noise level would make it very difficult to derive small temperature changes resulting from chemical or physiological processes beneath the surface.

Figure 6B ("bottom read") shows the range of measured temperatures as a function of time, after subtraction of common-mode noise, reading through the infrared-transparent film. The data show significantly reduced temporal noise, with temperature variations of less than about 0.025°C (PTP). In this case, the film reduces or prevents evaporative cooling because the fluid no is longer exposed to dry air . This reduction in evaporative cooling reduces noise in the measurement, which allows the system to record significantly smaller changes in temperature. This measurement technique, particularly when combined with the novel multiwell plate design of Figures 2 and 3, allows accurate recording of small subsurface processes taking place in the sample well, improving measurement resolution by about fourfold.

Example 5

Figure 7 shows results of an experiment designed to determine the preferred number of frames to average in the frame-averaging noise-reduction technique. The experiment was performed using top-read thermal-imaging apparatus and low cross-talk multiwell plate of Examples 2-4. The experiments show the noise level at several areas of the multiwell plate, averaged over 0, 2, 4, 8, and 16 frames at 60 Hz. The following chart summarizes the data for selected areas within the image:

	Normal	2× Frames	4× Frames	8× Frames	16× Frames
Max (bit counts)	10656.00	10632.00	10632.00	10616.00	10600.00
Min (bit counts)	10560.00	10576.00	10584.00	10576.00	10568.00
Avg (bit counts)	10611.17	10604.05	10610.28	10592.24	10582.40
Range (Kelvin)	0.60	0.35	0.30	0.25	0.20
Std Dev (Kelvin)	0.10	0.07	0.05	0.04	0.04

Values are in 14 bit units. The "std dev" row is the root-mean-squared (RMS) noise of a uniform target after application of frame averaging.

Example 6

Figure 8 shows results of an experiment designed to determine the level of common-mode noise typical of an infrared camera. The experiments were performed using the apparatus and plate of Examples 2-5. The figure shows a typical raw data set after frame averaging (high-frequency noise reduction) and area averaging, but before common-mode noise reduction and offset subtraction.

Example 7

Figure 9 shows results of an experiment designed to assess the ability of offset subtraction to extract data for a 135-μW reaction in a multiwell plate. The experiments were performed using the apparatus and plate of Examples 2-6.

Figure 9A shows raw data for an experiment using four sample wells in which one well received a "sample" comprising a constant 135-μW input, while the other wells received a benign (i.e., non-reactive) sample. The data show the average temperature in the measurement region as a function of time, after image averaging is applied.

Figure 9B shows the same data after offset subtraction, which adjusts the data so that each measurement starts at zero at time zero. There is a residual common-mode noise

of approximately 0.05°C PTP, after offset subtraction. This common-mode noise may be out of phase and may shift depending on the geometric position of the cell, as shown in Figure 8.

Example 8

Figure 10 shows results of an experiment designed to assess the ability of offset subtraction and the reduction of common-mode noise using a reference region local to the measurement region to extract data for the 135-μW reaction of Example 7 and Figure 9. The reduction of common-mode noise reduces the noise level for the benign wells to an RMS level of about 0.004°C. The presence and thermal profile of the 135-μW reaction is clearly visible relative to the benign wells. The data compare favorably to a thermal model that calculates the theoretical effect of a 135-μW input on a sample holder having the same fluid volume. Without using the measurement and noise-reduction methods described here, the reaction resulting from a 135-μW input could not be detected using an infrared camera.

Example 9

This example describes software for performing and/or evaluating calorimetric measurements.

Figure 11 shows a software screen for the display of thermal data. The software screen may include one or more data presentation fields. These fields may be used to presentat data using any suitable form, including tables, graphs, and pseudo-images, among others. The fields may include a single display that includes or summarizes data

from multiple samples, and/or multiple displays that each include or summarize data from one or a subset of the multiple samples. If there are multiple displays, they may be arranged in a manner representative of the layout of the corresponding samples, such as an 8×12 array of mini-graphs corresponding to the 8×12 array of samples in a standard 96-well microplate. The data displayed in the software screens may include a characteristic of the thermal radiation detected, such as the amount, intensity, and/or spectrum of the radiation. The data also may include a computed quantity related to a characteristic of the thermal radiation detected, such as a temperature. The data also may include kinetic data, such as temperature versus time (denoted "tick"). The software screen also may include software switches for selecting the scale of the display, for example, X-axis and Y-axis scales for graphical data and color schemes for pseudocolor images, among others.

Figures 12 and 13 show software screens for collecting, displaying, and/or calibrating data relating to the measurement and reference regions. The top screen shows the application screen for collecting data using the circular reference around the perimeter of the measurement region. The bottom screen shot shows the setup window for defining the characteristics of the reference. These screens again may include one or more data presentation fields and one or more software switches, among others. Moreover, these screens may permit recording and/or reporting of system parameters, such as emissivity, object distance, ambient temperature, relative humidity, noise reduction methods, and/or sample holder format, among others.

Example 10

This example further describes noise-reduction methods provided by the invention.

The noise-reduction methods may include (1) converting detected thermal infrared radiation to a signal, and (2) processing the signal to reduce the proportion of the signal that is attributable to noise. The step of processing the signal may include a step of temporally averaging the signal comprising computing a quantity based on distinguishable components of the signal representing thermal infrared radiation detected from the same sample at different times. Alternatively, or in addition, the step of processing the signal may include a step of spatially averaging the signal comprising computing a quantity based on distinguishable components of the signal representing thermal infrared radiation detected from different portions of the same sample.

The noise-reduction methods also may include (1) detecting thermal infrared radiation transmitted from a plurality of samples contained in the sample wells using an optical device, (2) converting the thermal infrared radiation detected from each sample to a corresponding signal, and (3) adjusting the signals so that each has the same preselected value at the same preselected time. The preselected value may be zero, among others, and/or the preselected time may be zero, among others.

The noise-reduction methods also may include (1) detecting thermal infrared radiation transmitted from a reference region adjacent a sample, and (2) constructing a sample signal characteristic of the thermal infrared radiation detected from the sample based on the thermal infrared radiation detected from the sample and the adjacent

reference region. The reference region may comprise an annular portion of the sample plate distributed adjacent a perimeter and/or about a central or optical axis of the sample well.

The disclosure set forth above encompasses multiple distinct inventions with independent utility. Although each of these inventions has been disclosed in its preferred form(s), the specific embodiments thereof as disclosed and illustrated herein are not to be considered in a limiting sense, because numerous variations are possible. The subject matter of the inventions includes all novel and nonobvious combinations and subcombinations of the various elements, features, functions, and/or properties disclosed herein. The following claims particularly point out certain combinations and subcombinations regarded as novel and nonobvious and directed to one of the inventions. These claims may refer to "an" element or "a first" element or the equivalent thereof; such claims should be understood to include incorporation of one or more such elements, neither requiring nor excluding two or more such elements. Inventions embodied in other combinations and subcombinations of features, functions, elements, and/or properties may be claimed through amendment of the present claims or through presentation of new claims in this or a related application. Such claims, whether directed to a different invention or to the same invention, and whether broader, narrower, equal, or different in scope to the original claims, also are regarded as included within the subject matter of the inventions of the present disclosure.